ANATOMY OF THE YOUNG VEGETATIVE SHOOT OF *TAKHTAJANIA PERRIERI* (WINTERACEAE)[1]

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ABSTRACT

Material of the rare monotypic genus *Takhtajania* has been examined for anatomy of the young vegetative shoot. Leaf cuticles are smooth, stomata mostly brachyparacytic and free of alveolar deposits common to other Winteraceae. Mesophyll structure is weakly differentiated. Petiole bases are vascularized by three independent traces, each of which may form pairs at the nodal level. Splitting of lateral traces leads to a more or less 5-trace V-pattern at higher levels of the petiole. Young stems are pseudosiphonostelic and have little sclerenchyma. Within the family, *Takhtajania* appears among the least specialized genera, with *Drimys* and *Tasmannia* coming closest histologically. Among other ranalian families, Canellaceae appear to be nearest this level of specialization, mostly on the basis of similarities in stelar and nodal structure.

Key words: Canellaceae, leaf, nodal anatomy, stem, *Takhtajania*, vegetative shoot, Winteraceae.

Phylogenists have long considered Winteraceae to be among the least specialized in the angiosperms (cf. Takhtajan, 1997). Among analyses supporting this conclusion are those based on the presence of vesselless wood (Carlquist, 1983; van Tieghem, 1900), a mixture of sieve-element plastids (Behnke, 1988; Behnke & Kiritis, 1983), plesiomorphic floral structure (Erbar & Leins, 1983; Endress, 1983; Vink, 1988), and paleoantarctic distribution (Smith, 1945). *Takhtajania*, the rarest and most recently recognized genus, has been known only from dried type material. It is isolated from other Winteraceae geographically and morphologically. Only recently has liquid-preserved material been available in support of a detailed study of its morphology and anatomy. The most important questions to be initially considered were (1) what is the place of the genus among its likely neighbors, and (2) what is its level of specialization within the woody Ranales.

The original *Takhtajania* collections were made in Madagascar in 1909 by Perrier de la Bâthie. They awaited examination at Paris (P) until Capuron (1963), finding them to represent a new taxon of Winteraceae, named the collection *Bubbia perrieri*. Later study of the leaves by Baranova led to reevaluation of *B. perrieri* and its recognition as the monotypic genus *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy. At the same time, it was placed in a new subfamily Takhtajanioideae by Leroy (1978). The species remains geographically isolated from other known genera of the family.

After repeated attempts over the past 25 years to find more material, Malagasy parataxonomist Fanja Rasoavimbahoaka located a second population of *Takhtajania* in 1994 about 150 km east of the original locality. Then in spring 1997, the new locality was revisited by parataxonomist P. J. Rakotomalaza and Chris Birkinshaw who collected liquid-preserved flowering and vegetative material. For more detailed information regarding the interesting collection history of this taxon, see Schatz (2000 this issue).

At the Missouri Botanical Garden, the preserved materials were divided among several investigators who are contributing observations on wood and reproductive organs. This contribution includes observations on leaf vasculature from clearings and transsections of the leaf lamina and midrib, petiole and nodal anatomy, and other anatomy of the young vegetative shoot.

MATERIAL AND METHODS

Specimens of vegetative and flowering material were collected in the Anjanaharibe-Sud Special Reserve southwest of Andapa in northeastern Madagascar as follows: *P. J. Rakotomalaza et al. 1342*, and *C. Birkinshaw 483*, 14°45′S, 49°28′E, at 1200 m altitude in a perhumid forest with abundant moss and lichens covering the stems.

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Young stems and leaves used in this study were field preserved in 50% ethanolic FAA and transferred to 70% ethanol upon arrival at the Missouri Botanical Garden. Leaf clearings were made by an improved method (Keating, unpublished) where specimens were placed in cold (room temperature) 5% NaOH and then microwaved. The 750 watt magnetron was set on defrost cycle, i.e., pulsed power, 2 seconds on/off for three cycles of 20 seconds. This promoted uniform clearing and avoided boiling, which is quite destructive of soft tissues. The NaOH-covered specimens were then placed in a 45°C oven for 48 hours during which time clearing was completed. Specimens were immersed in cold (room temperature) 5.25% sodium hypochlorite (Clorox®) for 30 minutes until the specimen was white. After three gentle tap water rinses, the specimen was soaked in I_2KI (0.2/2.0%) (v/v) in 15% ethanol for two hours until uniformly brown. Without further aqueous rinse, the specimen was changed to 20% $CaCl_2$ solution and then mounted. Within minutes, dark magenta veins contrasted against a transparent background in the cleared specimens.

Cross sections of leaf lamina and midrib, petiole, node, and young stem were made by hand microtome as well as free-hand and mounted in $CaCl_2$. Some sections were mounted unstained in glycerine. Most were stained in cresyl violet acetate, toluidine blue, Schiff's reagent, or I_2KI. Differentiation of these dyes is sharp in the strongly ionic $CaCl_2$ (Herr, 1992; Keating, 1996). Two additional specimens of liquid-preserved young stems with attached leaf bases were paraffin-embedded, serial-sectioned transversely on a rotary microtome at 10 μm, and stained in Safranin-O and Fast Green FCF.

Observations

Shoot Apex

The young stem expands massively within 5–7 mm of the shoot tip causing the apical meristems to appear somewhat embedded among massive petiole bases (Fig. 1A, B). From an early stage, petiole bases show an obvious cicatrice constriction (Fig. 1A). Young primordia form a 2/5 phyllotaxy of petiole bases that show early cell division and expansion, laterally and dorsiventrally. Packing geometry causes petiole bases to remain partially fused at the shoot apex level and to form polygonal shapes (Fig. 1C). The axillary bud is, in two observations, an outgrowth of the adaxial side of the petiole of the 7th primordium (Fig. 1D). No vascular connections could be followed, nor was any bud development visible in the true axil of more mature nodes.

Leaf Surface and Paradermal Anatomy

Leaf outline elliptical (Fig. 2A) with a decurrent base (Fig. 2B). Length/width ratio 4–5:1. Cuticle smooth. Epidermis: cells rounded-polygonal (1–3: 1 l/w) (Fig. 2C). In some areas of older leaves cuticular flanges form thick dividers between cells, 3–7 μm wide. Stomata abaxial only, guard cell pairs with lengths 22–29 μm, widths 18–21 μm, most commonly brachyparacytic with four adjacent subsidiary cells that differ only in position from normal epidermal cells (Fig. 2C). Occasionally ca. 20% of stomata have 5 or 6 subsidiary cells, but remain brachyparacytic with regard to the presence of two epidermal cells always parallel with the guard cells. Mesophyll: cells of adaxial palisade zone appearing compact and shortly lobed with ca. 10% air cavity space. Spongy tissue with numerous air cavities 2–3× as broad as cell diameters. Air space ca. 60%. Venation pinnate, looped brochidodromous. Intercostal areas irregular. Higher-order venation showing incomplete and very irregular areolation (Fig. 2D), 0–6 vein endings per areole (Fig. 2E). Areole sizes: smaller ones ca. 340 μm isohedral; larger ones ca. 2610 × 1248 μm.

Leaf Transsection

Structure weakly dorsiventral (Fig. 3A). Midrib: outline shallowly concave adaxially, deeply convex-rounded abaxially. Cuticle smooth, thickness 5–7 μm (Fig. 3B). Cuticular flanges absent or narrow between epidermal cells, up to 18 μm deep. Epidermis: cells thin and cuboidal on both surfaces. Stomata level with surface. Guard cells small, oblique to and oriented toward the surface in relation to adjacent subsidiary cells, which subtend the guard cells internally (Fig. 3D). Outer cuticular ledge well developed at stomatal opening; internal ledge not present or much smaller. No cuticular alveolar deposits occlude the stomatal openings. Hypodermis generally absent although adaxial-most layer of mesophyll may lack chloroplasts in some areas. Mesophyll differentiation weak. Cells of the palisade zone mostly small, rounded cells in 1–3 closely packed layers, occupying up to 25% of mesophyll, forming a gradual transition to spongy tissue. Spongy cells in lower mesophyll horizontally elongated with irregular long lobes. Chloroplasts most densely packed in adaxial mesophyll cells, gradually becoming more dispersed in abaxial mesophyll. Air cavities: most larger cavities abaxial and aligned over stomata as deep substomatal cavities extending above the center of the mesophyll (Fig. 3A, D).

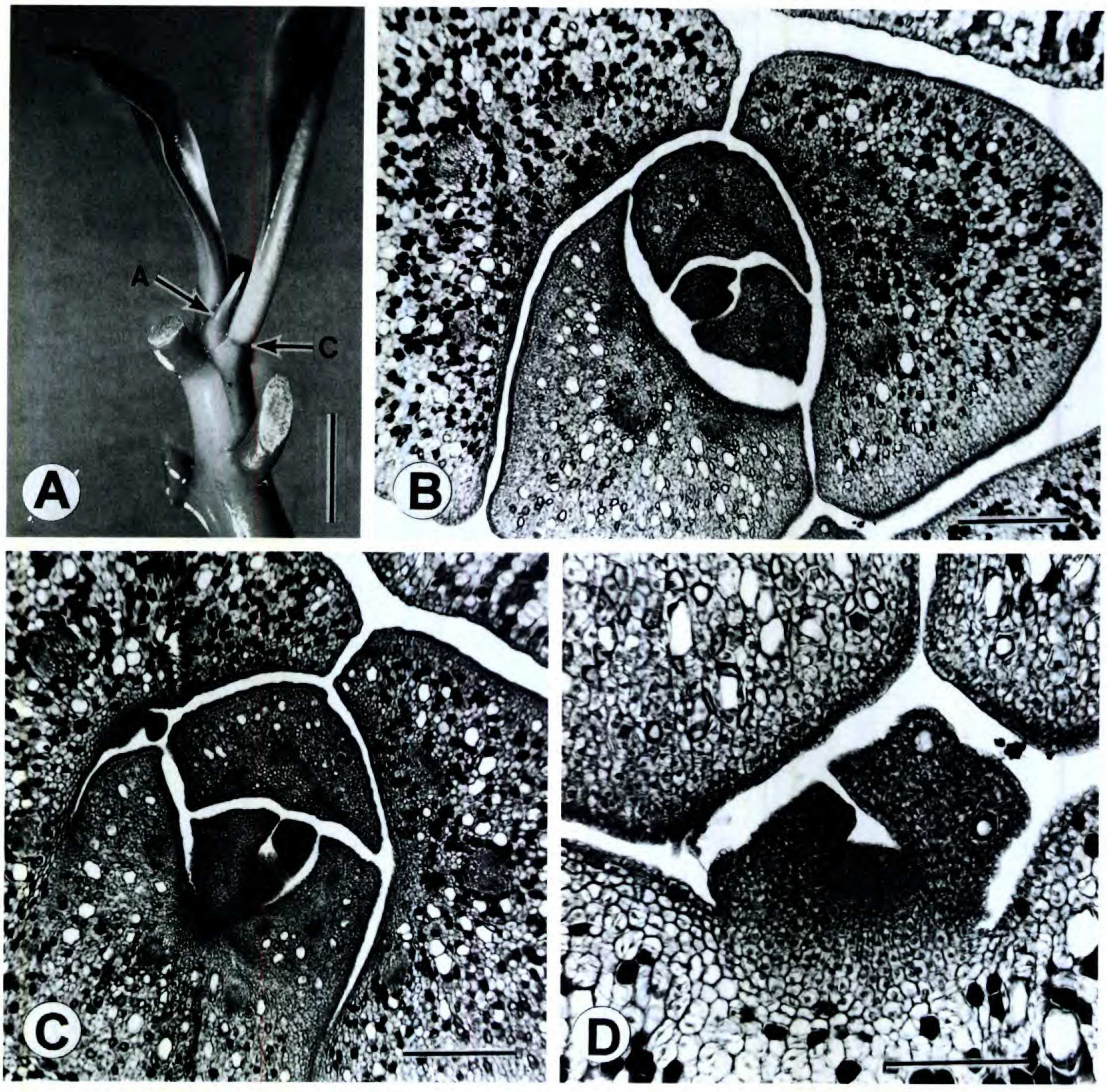

Figure 1. *Takhtajania perrieri* shoot tip and apical meristem region. —A. Young shoot tip showing apical meristem region (A-arrow) and cicatrice constrictions (C-arrow). —B. Transsection of young stem tip, ca. 100 μm distal to apical meristem. Base of leaf primordia determined by packing geometry. —C. Shoot apex at apical meristem level. Bases of several leaf primordia fused as meristem is partially sunken at shoot summit. —D. Shoot apex ca. 120 μm distal to apical meristem. Axillary bud attached to leaf primordium base. Scale lines: A = 1 cm; B, C = 300 μm; D = 150 μm.

Midrib vasculature usually 5 bundles arranged in a V-shape, one abaxial bundle and 2 or 3 lateral bundles on each side. Xylem: largest bundles occur with 10–12 files of metaxylem facing phloem with a convex procambial boundary. Phloem: crescent-shaped strand with weak or no visible differentiation of sieve elements and companion cells. Cells show no alignment or regularity of pattern in cross section. Bundle sheaths a single layer of thin-walled cells, little modified from spongy tissue. Sclerenchyma: lateral-most bundles in midrib have 1–2-layered extraxylary fiber cap outside phloem. Secondary bundles with two lateral fiber strands at phloem and small strand capping xylem. Secretory tissue: slightly enlarged oil cells frequent in mesophyll (Fig. 3C, E). Tannin cells in midrib area in discontinuous row bounding phloem; some dark smaller cells within phloem. Starch grains either common throughout cells of the lamina, or grains mostly in spongy cells and not numerous. In midrib, starch grains packed in ground tissue internally adjacent to xylem points. Grains less common but often well developed in all ground tissue in vicinity of vascular strands.

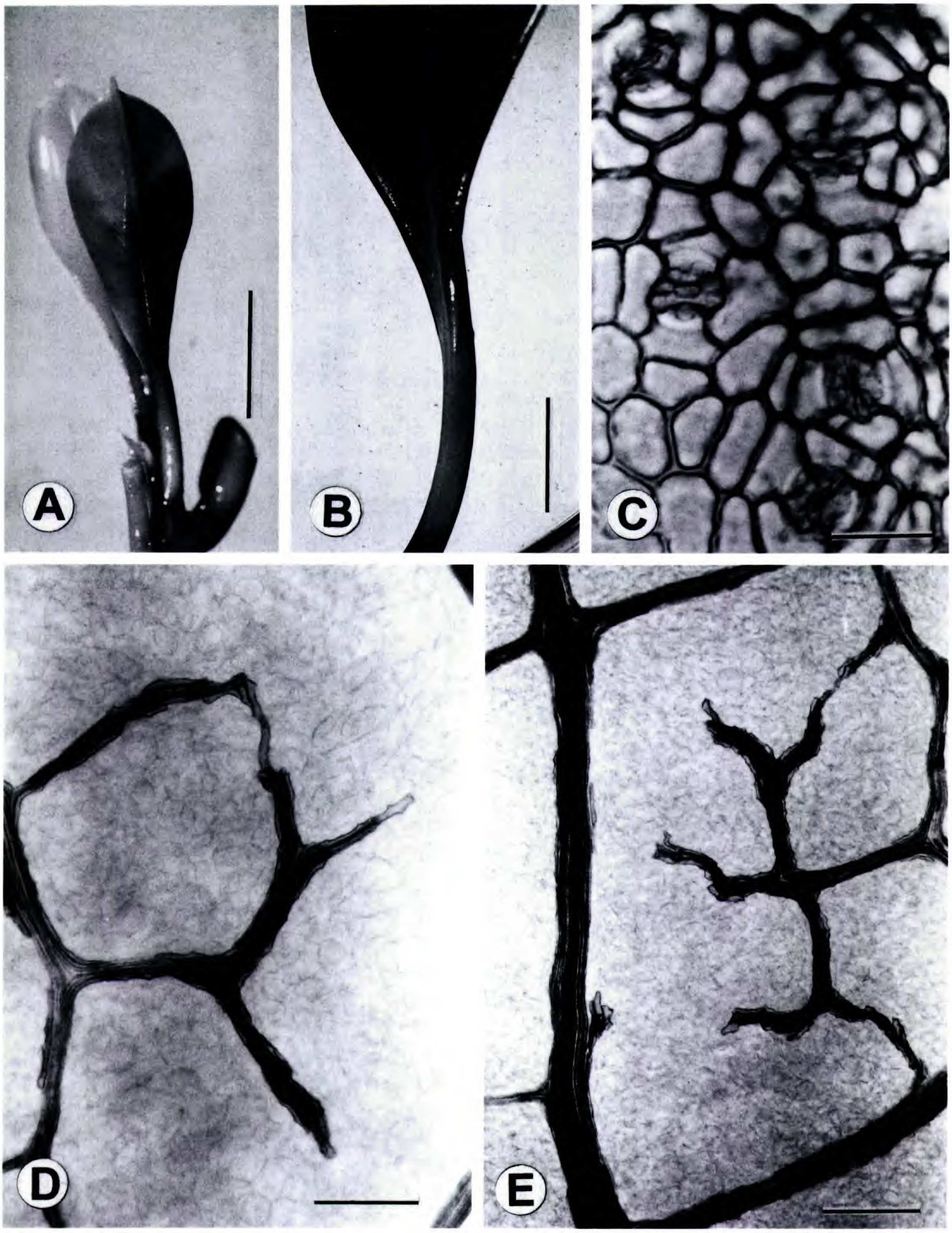

Figure 2. *Takhtajania perrieri* young leaf structure. —A. Pair of young leaves, not actually opposite, at shoot apex. —B. Young leaf, adaxial surface showing decurrent lamina. —C. Abaxial surface of epidermis showing polygonal epidermal cells and stomata. —D. Leaf clearing showing small areole and free vein endings. —E. Leaf clearing showing large imperfect areole and free vein endings. Scale lines: A, B = 1 cm; C = 50 μm; D = 200 μm; E = 300 μm.

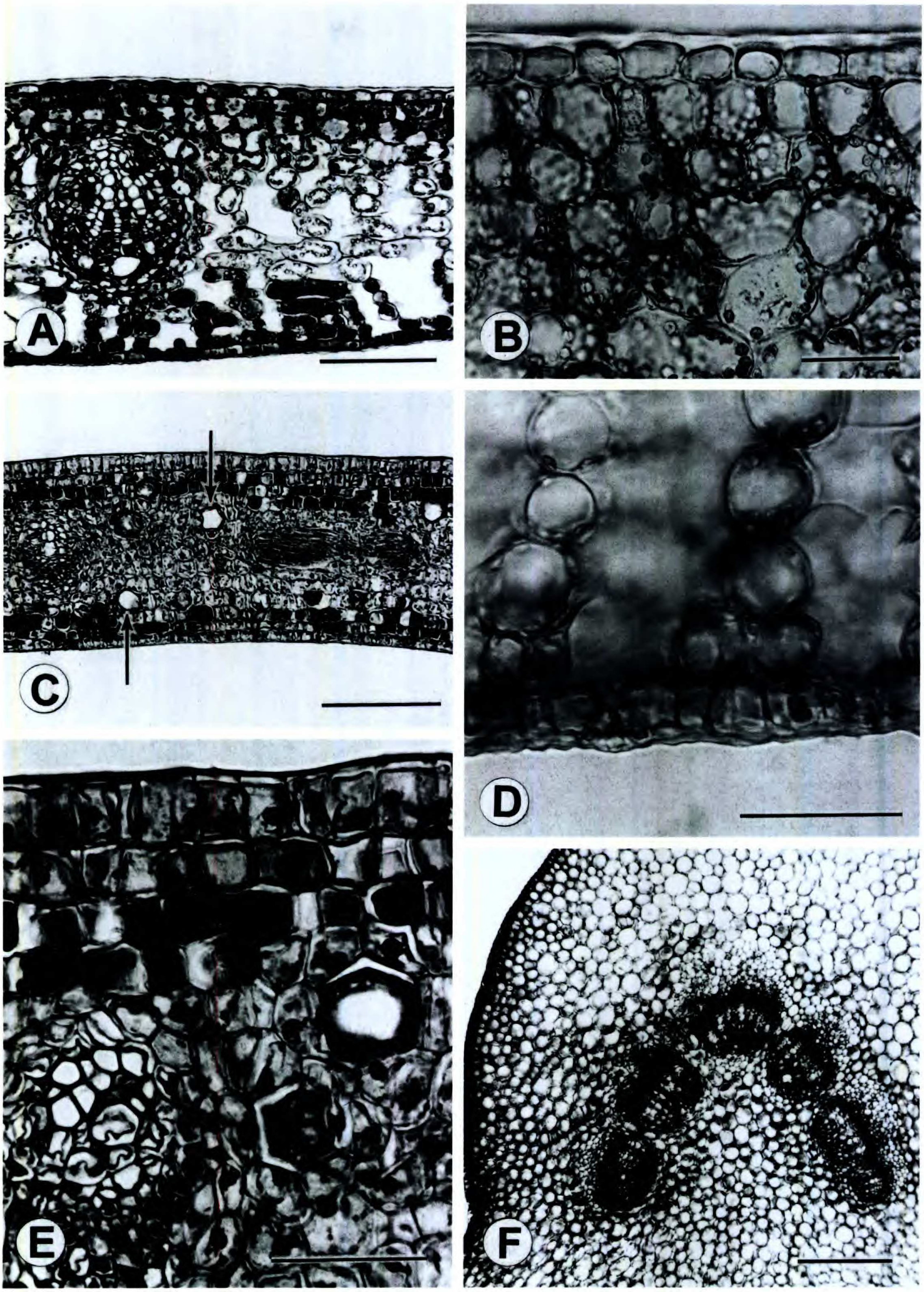

Figure 3. *Takhtajania perrieri* leaf and petiole structure. —A. Lamina transsection (TS) showing compact mesophyll in palisade region and circular vascular bundle. —B. Lamina TS, hand section showing smooth thick adaxial cuticle. —C. Young lamina before mesophyll expansion showing several mature oil cells (arrows). —D. Lamina TS abaxial surface. Stomata with guard cells arranged obliquely to epidermal cells. —E. Young lamina showing young oil cells. —F. Petiole base showing V-pattern of median bundle and divided lateral bundles. Scale bars: A, D = 150 μm; B, C, E = 50 μm; F = 300 μm.

Figure 4. *Takhtajania perrieri* stem and nodal structure. —A. Young stem TS outer cortex, epidermis, and cuticle. —B. Stem TS pith and starch grains. —C. Node showing median trace and two lateral traces. —D. Node TS hand section showing split median trace and one lateral trace. —E. Node TS divided median trace within the stele. Small

PETIOLE TRANSSECTION

Outline: flattened adaxially at base, becoming terete distally. Size: diameter 2.5 mm. Cuticle smooth, thickness 3.6–7 μm. Epidermis: cells small and cuboidal, diameter ca. 22 μm. Ground tissue: cells spheroidal, diameter 26–70 μm, loosely packed but with no organized aerenchyma. Intercellular air spaces at cell corners comprising 10–20% of ground tissue. Venation: 3 to 7 collateral vascular bundles arranged in V-shape (Fig. 3F). Xylem: several protoxylem points lead to organized files of metaxylem, up to three cells wide per protoxylem point. Xylem production ends at straight or broadly convex cambial boundary with hemielliptical phloem strand. Phloem: sieve elements and companion cells in tiers 1–2 cells wide. Tannin cells abundant in ground tissue, these not differentiated from ground tissue except by dark-staining contents. Tannin cells randomly scattered abaxially or in radial rows adaxially. Starch grains common in cells adjacent to vascular bundles.

YOUNG STEM TRANSSECTION AND LONGISECTION

Outline terete. Diameter ca. 1 cm. Cuticle smooth, thickness, ca 3.5 μm (Fig. 4A). Epidermis: cells small and cuboidal. Chlorenchyma: chloroplasts very common in outer cortex. Cortex: ground tissue cells mostly spherical, loosely packed with numerous air cavities. Cells axially elongate, 2–3× cell diameter in transsection. Air space ca. 20% of cortex. Pith cells polygonal and somewhat larger than cortical parenchyma cells; air cavities 5–10% of pith. Vascular bundles collateral. Xylem: distinct protoxylem points at pith boundary but xylem quickly develops cambial growth producing a pseudosiphonostele within 90 μm of pith boundary. Phloem: sieve element arrangement irregular. Companion cells not easily distinguished in cross section. Widest pith rays with slight tendency toward ray dilation in phloem. In longisection, metaphloem sieve element length up to 395 μm. Early secondary sieve element length 215–290 μm. Sieve plates horizontal or 5–10° off horizontal and somewhat broadened. Companion cells short or mostly as long as sieve elements, highly variable in shape and size.

Sclerenchyma: no extraxylary fibers present in small stems. In one sample, ca. 9 mm diameter, occasional single fibers occur around phloem perimeter in inner cortex. In longisection, these single cells overlap and are contiguous for at least longer than 2 cm. In same larger specimen, sclerotic layer occurs subepidermally around perimeter. Sclerotic cells in 1–3 layers, heavily lignified, with up to 20 isotropic wall layers especially well developed on inner and outer periclinal walls. Tannin cells scattered and common in all ground tissue. Starch grains rounded, single or in clusters (Fig. 4B). Grain diameter 9–13 μm. Grains common throughout pith and in sheath of 5–10 inner cortical cell layers.

STEM–NODE–LEAF CONTINUUM

Stem outline in the vicinity of the node is irregularly elliptical. In the young stem, the vascular procambium appears to be a nearly continuous circle with maturation of proto- and metaxylem only observed in what will become leaf traces. A characteristic cut through the node of a stem with a young active cambium shows three non-adjacent traces vascularizing the leaf base, i.e., the node would be called three-trace, tri-lacunar (Fig. 4C) using the original siphonostele paradigm begun by Jeffrey (1898) and carried into dicot nodal anatomy by Sinnott (1914). However, the two specimens observed here show some intriguing differences. The older stem sections show most leaf traces appearing double, that is, there are two proto-xylem points causing the development of two paired traces. They may cause a node to appear 2:2:2, 2:2:1, or 1:2:1 (Fig. 4D, E, F). In the younger stem pieces, which were paraffin-sectioned at 10 μm, this observation was confirmed less frequently. Diagramming the vasculature through several nodes showed mostly late-appearing bifurcations of larger bundles.

Nothing was found to indicate that the individual leaf traces, especially the median trace, have components that occur in different sympodia of the vasculature. In this material, leaf traces were well defined through at least four nodes before entering a leaf base. New leaf traces appeared to arise from undifferentiated interfascicular procambium. More material, sectioned at closer intervals, will be needed to identify vein sympodium branching patterns. Following the leaf base distally from the node, the three traces are observed to relate in a V appearance, whether or not they appeared as double at

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incipient traces visible within the procambial cylinder. —F. Node TS showing divided lateral traces at level of procambial cylinder. Scale bars: A, B = 50 μm; C, D = 300 μm; E = 200 μm; F = 150 μm.

the nodal level. Distally, in the leaf base, the two lateral traces bifurcate to form two pairs of lateral traces. Using Sugiyama's (1979) notation the *Takhtajania* node can be most usually figured (moving distally into the petiole) as L-M′M-L ⇒ L-M-L ⇒ L′L-M-L′L. In the base of the petiole and continuing up to the lamina, the resulting five traces form a marked V of closely associated vascular bundles with the median trace forming the base of the V.

Axillary buds do not appear among the first 4–5 leaf primordia. They occur removed from the axil, high on the adaxial side of young petioles (Fig. 1D). No vascular connections to axillary buds were detected in this material.

Discussion

THE LEAF

Leaf venation is typical of other ranalian families with elliptic leaves with entire margins. The pinnate, festooned brochidodromous structure, accompanied by imperfect and variable areole structure, produces a low first-rank leaf (cf. Hickey, 1977: 158).

Metcalfe (1987) noted much variability in cuticle texture and sculpturing in Winteraceae, and *Takhtajania*'s smooth, unsculptured cuticle seems diagnostic within the family. Bongers (1973) noted that leaf cuticles of *Takhtajania* and some *Drimys* species lack the alveolar layer present in all other Winteraceae. However, Baranova (1972) noted that "Group 2" Winteraceae, *Bubbia*, *Belliolum*, *Pseudowintera*, and *Zygogynum*, also have ordinary (= non-alveolar) or more or less grainy cuticles. Existing data are not a useful guide to relationships until comparable observations can be made across the genera. The known leaf venation trends are not sufficiently refined for one to offer an opinon on whether *Takhtajania* is more or less specialized than other winteraceous species.

Leaf surface observations do not confirm Baranova's (1972) report of the existence of mostly anomocytic stomata in *Takhtajania*; instead, stomata are mostly brachyparacytic as is true of other winteraceous genera. This removes a major argument for subfamily-level segregation of the genus. Also, stomatal apertures are said to be occluded with alveolar material (Metcalfe, 1987; Bongers, 1973; Baranova, 1972), but this is not true in *Takhtajania* or in *Drimys* (*Tasmannia*) *piperita* Hook. (Bongers, 1973).

In genera studied thus far, including *Takhtajania*, mesophyll in the family is reported to be not clearly differentiated into palisade and spongy mesophyll, e.g., *Exospermum* (Carlquist, 1982) or *Zygogynum* (Sampson, 1983). This weakly bifacial structure is consistent with a relatively low placement of this leaf structure among the Ranales. Bailey and Nast (1944b) noted that thicker, more coriaceous leaves in *Drimys* have large sclereids in the mesophyll between vascular bundles, and Rao and Das (1979) also reported nests of sclereids in the family. By their absence, *Takhtajania* shows simpler structure. In most Old World genera of Winteraceae, all foliar vascular bundles are commonly ensheathed by a slender ring of thick-walled sclerenchyma cells. This was not observed in *Takhtajania*.

THE NODE

Observations on nodal anatomy are not as systematically powerful as they could be, partially due to persistent use of the siphonostele paradigm (see review by Beck et al., 1982), partially to unsolved theoretical concerns regarding the reality of cauline vasculature, and partially to the difficulties in relating nodal traces to stelar vasculature. At least for three-trace ranalian genera showing open architecture, the following discussion predicates that vascular patterns are best recognized as a cylinder of eustelic sympodial bundles of varying number. Followed from a proximal to distal direction, all of these bundles become leaf traces at regular intervals. In terms of their origin, all so-called cauline bundles are first identified as leaf traces. They probably connect basipetally to existing leaf trace sympodia.

As seen from the contiguous older nodes and two short pieces of stem tip available in the present study, nodal structure places *Takhtajania* among Benzing's (1967) trilacunar ranalian families. Benzing noted that nodes in *Bubbia* sp., *Drimys colorata* Raoul, *D. winteri* J. R. Forst. & G. Forst., *Canella alba* Murray, and *Warburgia ugandensis* Sprague are so similar that they can be described as one. All five species showed a 2/5 phyllotaxy, which is also confirmed in *Takhtajania*. Benzing's specimens showed distinct helicoid sympodia where every 5th leaf is supplied by a median trace from the same sympodium. This seems possible but could not be quite confirmed from currently studied *Takhtajania* material. The relationship of 3 traces vascularizing a leaf to similar sets of traces above and below that node appears somewhat unpredictable and irregular in the available material (Fig. 5).

About 5 mm below the apical meristem, in a region of closely spaced nodes, the vasculature of the young stem forms a eustele consisting of precociously matured xylem strands, here interpreted

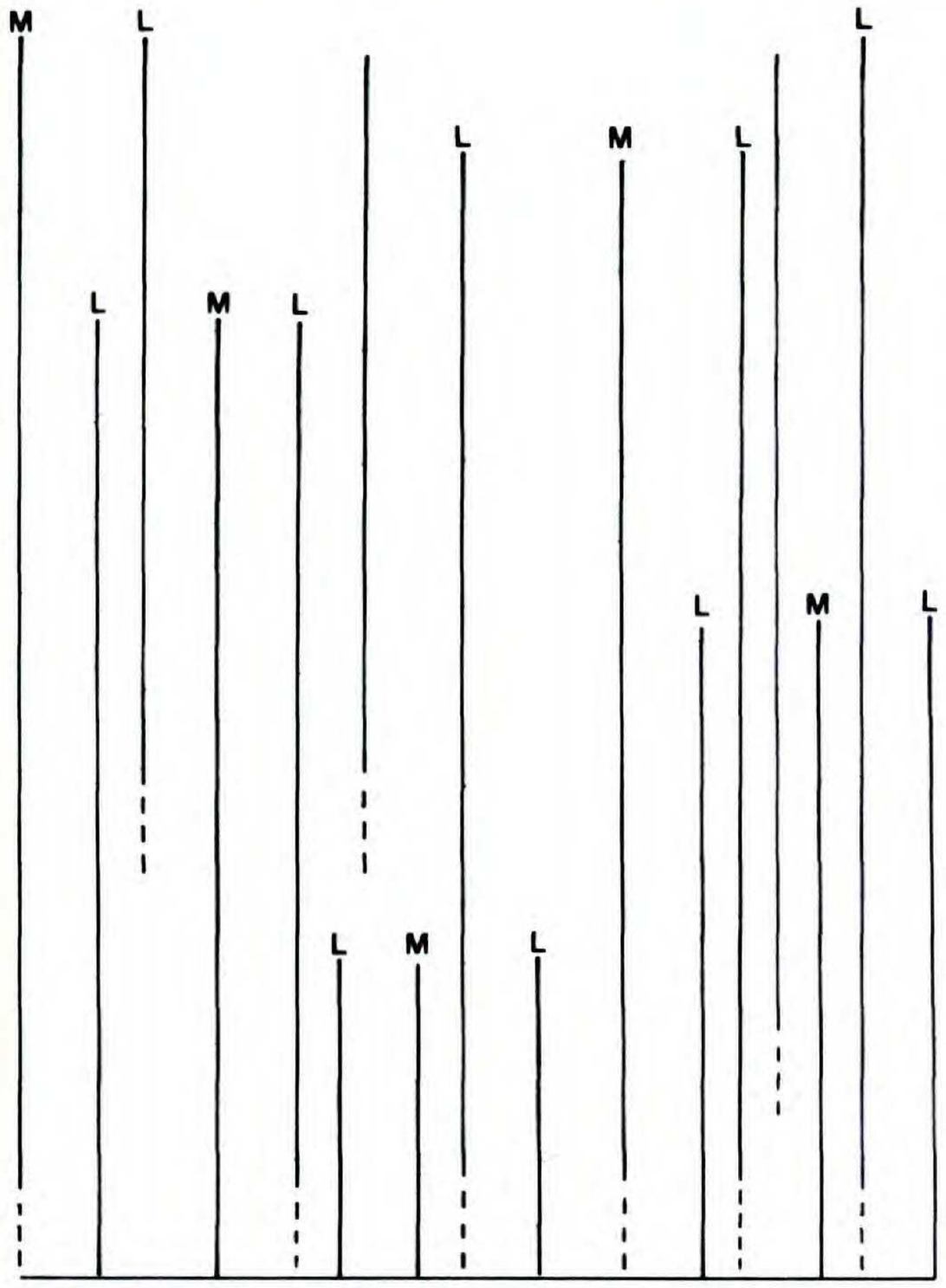

Figure 5. *Takhtajania perrieri*: diagram of shoot vascular pattern through five nodes, reconstructed from 10 μm serial sections. Eustelic bundles appearing as solid lines, each eventually becoming a leaf trace. Incipient traces occurring entirely within cambial zone shown as broken lines. M = median leaf trace; L = lateral leaf trace. Other incipient traces, 2–5 between larger traces, are not shown. Leaf trace bifurcations occurring at various levels also not shown.

as leaf traces, embedded in a cylinder of procambium. Usually, the clearly defined vascular bundles occur in pairs or threes, rather than as singles. The vascular diagram for *Takhtajania* (Fig. 5) shows that lateral and median leaf traces arise independently. All of the closely associated trace pairs do not arise from different sympodia as far as can be followed. All are superficial bifurcations arising distal to their identity as single incipient traces in the procambial cylinder.

An urgent need is the diagramming of the vasculature pattern from sections in the 1–3 μm range to see if the type of sympodial arrangement of terminal traces and renewal branches drawn by Benzing (1967) for a number of ranalian families can be confirmed. From the three short stem pieces available for this study, such an integrated pattern cannot be drawn.

As noted above, several relevant studies have to be translated from the cauline vasculature concept. In Bailey and Nast's (1944a: 215) study of the Winteraceae, they suggested that foliar bundles arise from "interfascicular parts of the hypothetical, cauline, primary vascular cylinder." Also, Beck et al. (1982: 795) stated, "There are 5 protoxylem strands in *Drimys winteri* that apparently represent axial bundles . . . ." Both of these conjectures cannot be related to the present observations. In *Takhtajania*, only leaf traces can be discerned when following vasculature through 4–5 nodes. There are no sets of 5 protoxylem strands at any level.

Beck et al. (1982: 795) also stated that, while there is much "variation in the mode of lateral trace production in *D. winteri*, one lateral trace typically diverges from the same axial bundle as the median trace, the other lateral may, ultimately arise from an adjacent axial bundle." No such pattern in *Takhtajania* can be observed or interpreted this way. In a similar line of reasoning, Howard (1974) noted that some workers refer to cauline bundles as those that persist in the stele through several nodes. This definition appears somewhat arbitrary since leaf traces, followed through several nodes, are still leaf traces, at least in these ranalian genera with open vasculature.

Nothing resembling the above scenario occurs in *Takhtajania*, nor does it agree with Benzing (1967). In that work, it can be seen that the essence of vascular sympodia is that all so-called cauline bundles are leaf traces, and the number of nodes along the way between a bundle's origin and its orientation into a petiole base does not change this definition. It would help if trace architecture of the ranalian shoot were interpreted consistently in terms of the primary vascular system in order to avoid artificial concepts and terminology (Esau, 1965).

The present observations of a pair of traces related to the median gap are a first report for Winteraceae, nearly fulfilling Takhtajan's (1964, 1969, 1991) conjecture of a hypothetical nodal type, which he believed to be ancestral for the angiosperms. But Takhtajan's prediction of a 1:2:1 set of traces varies from *Takhtajania*'s potential for having pairs at the lateral gaps also (2:2:2, 1:2:2). Swamy (1949) demonstrated pairs of bundles present in the cotyledonary node of *Degeneria* (1:2:1) and *Magnolia grandiflora* L. (pairs of median bundles and split laterals), but those taxa have multilacunar mature nodes. Observations by Marsden and Bailey (1955) on *Clerodendrum*, and by Bailey and Swamy (1949) and Dickison and Endress (1983) on *Austrobaileya* described double leaf traces where the components arose from independent portions of the eustele, a condition not found in *Takhtajania*. Other observations by Canright (1955) on Magnoliaceae and by Ozenda (1949) on *Liriod-*

endron, *Uvaria*, and *Anona* described three-trace or multitrace nodes where the median traces bifurcate or branch, tendencies also absent in *Takhtajania*.

In Sugiyama's (1979) broad study of Magnoliales nodal anatomy, *Drimys* (*Tasmannia*) *piperita* was diagrammed. Its three-trace node has a median trace that produces two small lateral traces which then degenerate: L-M-L ⇒ L-mMm-L ⇒ 'LL-M-L'L. In *Takhtajania* there are no aborted lateral median traces, but, otherwise, patterns found in *Tasmannia* are closer than those in most other genera to the pattern found here. If one considers multiple independent nodal traces to represent the least specialized condition for the angiosperms, as do Ozenda (1947), Sugiyama (1979), Neubauer (1981), and I, then the Winteraceae are not close to the angiosperm basal lines in this character. Finally, as noted above, because of the different assumptions brought to past studies in nodal anatomy, early trends of specialization in the Winteraceae will remain tentative until one anatomical study across the family generates descriptions based on one current paradigm.

PETIOLE VASCULAR PATTERNS

Superimposed on the basic three-trace architecture, several genera of Winteraceae develop quite complex petiole vasculatures. Bailey and Nast (1945) proposed two independent, plausible trends of specialization in the *Drimys* node: (1) three strands leading to numerous derivative bundles, and (2) three strands fusing to form a single vascular arc in the petiole. The three traces found in *Belliolum*, *Bubbia*, *Exospermum*, and *Zygogynum* divide to form three abaxial bundles and "numerous smaller bundles irregularly arranged in an adaxial position" (Metcalfe, 1987: 6). This seems to be a development parallel with Bailey and Nast's (1945) first *Drimys* pattern. Likewise, Metcalfe (1987: 6) described the bundles as being "amphicribral, hippocrepiform or appearing as divided bundles with their xylem units facing one another." Carlquist (1982: 281) characterized such a pattern in *Exospermum stipitatum* (Baill.) Tiegh. petioles and midribs as having "peculiar circles of bundles" as well as nests of sclereids in the mesophyll, both characters not found in *Takhtajania*.

Dehay and Ghestem (1969) illustrate distal petiole vasculature for three genera of Winteraceae. *Exospermum*, with its circular or semicircular traces in an irregular pattern, and *Zygogynum*, with numerous traces in three layers, are both quite distinct. *Bubbia*, with its simple V of 7 bundles in the petiole, as well as some species of *Drimys*, appears closest to *Takhtajania*. Such a pattern fits close to the base of Bailey and Nast's (1945) proposed trends for the 3-trace node.

THE STEM

Stem transsectional histology is quite simple. The uniform ground tissue of the cortex and pith is unexceptional. The early development of interfascicular cambium quickly produces an uninterrupted cylinder of cambial derivatives. This was termed the pseudosiphonostele by Bailey and Nast (1948), and the condition is common in woody Ranales. On the pith side of this continuous cylinder, the protoxylem is circumferentially discontinuous. In *Takhtajania*, secondary xylem accumulation initially remains limited as the young stem retains a succulence through the 1 cm diameter stage, the largest material available in this study. (See Carlquist, this volume, for details regarding secondary vasculature development.)

One unusual histochemical feature involves tissue lignification. In the presence of metachromatic dyes in combination with the highly ionic calcium chloride mountant (see Herr, 1992; Keating, 1996), tissue sections of most higher dicots and monocots show marked differentiation of lignified tissues from surrounding ground tissue and phloem. In *Takhtajania*, the lignification process appears to be poorly bounded, spreading beyond the limits of xylem, fiber walls, and cuticles. Using several detection systems (cresyl violet acetate, Toluidine blue, Schiff's reaction, iodine-potassium iodide, or phloroglucinol) few purely cellulosic-hemicellulosic walls were found. There seems to be at least a modest amount of lignification on all cells except sieve elements.

SPECIALIZATION AND RELATIONSHIPS OF *TAKHTAJANIA*

Takhtajania lacks the complexity of histology found in other genera of Winteraceae that have been studied in detail. Its lack of cuticular ornamentation and alveolar stomatal plugs, lack of sclereid development and sparing formation of extraxylary fibers, absence of other histological specializations, as well as its geographic isolation suggest that the plant should be interpreted as plesiomorphic and not particularly close to other winteraceous genera. This is consonant with Vink's (1988) proposed relationships. In terms of relative specialization, it would appear that *Drimys* (which shows quite variable structure) and *Tasmannia* are likely closest neighbors to *Takhtajania*.

On the basis of their analysis of *rbc*L nucleotide sequences, Qiu et al. (1993) suggested that the

Magnoliidae have five major lineages with Magnoliales and Nymphaeales being the most derived. Further, within the Magnoliales, they found Canellaceae and Winteraceae appearing closely related. Conclusions by Wilson (1965) and Benzing (1967) supported recognition of similarities between these families based on comparative anatomy. However, characters in the Canellaceae are variously more and less specialized. Advancements include having distinctively different floral morphology and anatomy, particularly fusions in the androecium (Wilson, 1966). Vascular structure differences include the presence of vessels, considered more specialized than the vesselless condition. On the other hand, sieve elements of Canellaceae have highly oblique to vertically oriented end walls with multiple sieve areas (Wilson, 1965) and are much less specialized than those cells found in *Takhtajania* and other Winteraceae (Zahur 1959).

Some characters are compatible but inconclusive. Sieve element size ranges are about the same in both families (Wilson, 1965). Observations on sieve-element plastids (Behnke, 1988) record the two families as being variable with overlapping P-protein types. Wilson (1965) encountered the same difficulties in attempting to trace the obscure origins of nodal architecture in the young stem of *Canella* such as occur in *Takhtajania*. Bailey and Nast (1945) emphasized the relative isolation of Winteraceae when they noted that, while variation within the family is wide, there is no overlap with other ranalian families investigated. In this context, Canellaceae could be most closely related.

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